NTP Research Concept: Cholesterol and Lipid Modulating Agents: Toxicological Approaches to Assessing Complex Mixtures

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Nomination Background and Rationale

"Drinking water disinfection by-products: 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibition and developmental toxicity" and "Drinking water disinfection by-products: Interactive effects of antilipidemic agents and drinking water contaminants in producing developmental toxicity" were nominated to the NTP and CERHR for Toxicological Evaluation by a private citizen. These nominations were reviewed and approved by the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC) in June, 2009. Given the interrelationship of both of these ICCEC-approved nominations, they will be presented as one concept to the NTP Board of Scientific Counselors in the Fall of 2010.

This project encompasses two complementary toxicological aspects. (1) Testing, improving and validating toxicological approaches to inform cumulative risk assessment by using an *in utero* toxicology dosing paradigm and evaluating subsequent alterations in developmental endpoints; and (2) characterizing the toxicological outcomes of *in utero* exposure to mixtures of agents that affect lipid and cholesterol utilization in the fetus.

Background

Need for improved approaches to cumulative risk assessment

Although risk assessments have typically been conducted on a chemical-by-chemical basis; in the real world, individuals are exposed to a multitude of complex mixtures. The field of mixtures toxicology is emerging as an area of scientific and regulatory focus. Data is needed to support the appropriate application of mixtures approaches to cumulative risk assessments. Recent mixtures studies with endocrine active compounds have demonstrated that chemicals that target a common signaling pathway or tissue can contribute to dose additive toxicity[(1)]. This implies that chemicals present at concentrations below their respective no observed adverse effect level (NOAEL) can incrementally add to a "total mixture dose" that could potentially elicit toxicity. Furthermore, the National Academy of Sciences report "Phthalate and Cumulative Risk Assessment: The Task Ahead" identifies that cumulative risk assessments should consider:

> …[T]he health outcomes and not on the pathways that lead to them, whether defined as mechanisms of action or as modes of action. Multiple pathways can lead to a common outcome, and a focus on only a specific pathway can lead to too narrow an approach in conducting a cumulative risk assessment. Accordingly, the chemicals that should be considered for cumulative risk assessment should be ones that cause the same health outcomes or the same types of health outcomes, such as a specific set of effects on male reproductive

development, not ones that cause the health outcomes only by a specific pathway [2].

Therefore, the development of approaches to predict the toxicological outcomes of exposure to mixtures, be it from chemicals with similar modes of action or similar adverse outcomes, is necessary.

Contaminants in drinking water and drug polytherapy as examples of mixtures

There are two general and potentially overlapping situations where exposure to mixtures may happen; environmental exposure (e.g. via drinking water, air, consumer products etc.) and clinical polytherapy. Complex mixtures found in drinking water may contain ubiquitous environmental contaminants, such as phthalates, drinking water disinfection byproducts as well as bioactive pharmaceuticals. The individual levels of pharmaceutical agents present in water are not typically quantified, and are not thought to be sufficiently high to pose a risk [3]. However, it can be hypothesized that individual compounds that have toxicologically similar modes of action (be they from pharmacotherapy and/or environmental exposure) may contribute to a cumulative adverse effect.

Pharmaceuticals that have complementary modes of action may be used concomitantly (e.g. statins and cholesterol absorption inhibitors). Although the potential toxicity resulting from co-administration of these drugs may have been assessed in animals, these studies are often conducted using the clinical dose ratios of the individual components[4]. In addition, it may not be possible for regulatory authorities to ask for interaction studies on drugs/agents from different registrants where they are not prescribed together for a specific indication. Therefore, depending on the concomitant therapy administered and the dose levels assessed, the potential for dose- or response-additivity may not have been adequately determined.

The question of interactions among drugs as well as ubiquitous environmental contaminants (that may also include pharmaceuticals) is challenging to address with experimental testing. In the case of pharmaceutical agents, the potentiation of an adverse response may be more easily identified because of the extensive knowledge of mode of action (e.g. drugs that lower cholesterol, blood pressure or chemotherapeutics) and pharmacokinetics facilitates the prediction of potential interactions. Several classes of common environmental chemicals are known to target the same signaling pathways or tissues as pharmaceuticals (e.g. both phthalates and antilipidemic agents target the steroidogenic pathway via different mechanisms). One of the challenges of undertaking mixture studies *in vivo* is the selection of the appropriate adverse outcome and determination of how this may be addressed experimentally. The assessment of developmental effects in the rat can be undertaken readily in the short term, since windows of exposure/ dosing paradigm during pregnancy are less than 3 weeks in duration.

Drugs that affect cholesterol and lipid metabolism are among the most prescribed medications [3,5,6]. These drugs largely belong to three pharmacological/mechanistic classes, the fibrates, statins, and inhibitors of cholesterol absorption. Fibrates decrease

cholesterol levels by increasing high density lipoprotein levels and are peroxisome proliferator activated receptor-alpha (PPARα) agonists, although they can have varying affinities for other PPAR receptors (e.g. PPARγ) [7]. Interaction with these receptors is an activity shared by a number of environmental chemicals, including the phthalates, perfluoroalkyl sulfonates and carboxylates (PFAAs), and the water contaminants trichloroethylene and trichloroacetic acid [9]. Representatives of the statin and fibrate families have been found in wastewater in concentrations approaching µg/L concentrations and have been followed to some extent through treatments rendering the water suitable for potable reuse [10]. The phthalates and perfluorinated acids are seen in wastewater (10s of µg/L), but their potency as PPARα agonists are less than the fibrates [11,12]. The latter compounds are typically administered in doses ~50 mg/day and are found in wastewater at concentrations 100-2500 ng/L [13]. Perfluorinated acids occur in ng/L concentrations unless there is a significant local industrial input when concentrations can be found in the µg/L level [14].

The cholesterol lowering effects of the statins are attributed to the inhibition of HMG-CoA. This also appears to be the mechanism by which developmental toxicities are produced [15]. Toxicity induced in rats from gestation day 15 through weaning was readily reversed by supplementing the diet with the HMG-CoA reductase product mevalonic acid, supporting the hypothesis that these effects were attributable to inhibition of HMG-CoA[16]. Cholesterol plays an essential role as a cofactor in molecular signaling processes involved in differentiation, therefore, the statins are contraindicated in pregnancy [17]. Nevertheless, females with familial hypercholesterolemia may become pregnant while taking these therapies.

A common disinfection byproduct, dichloroacetic acid (DCA), also decreases HMG-CoA reductase activity when administered to rats and produces cardiopathic effects in the fetal rat [18,19]. Myopathies are one of the major adverse effects of the statins in animals. It is possible that the related dihaloacetic acid byproducts may share these effects at high doses.

Key Issues

Assessment of chemical interactions requires carefully designed studies where each chemical can contribute to the mixture effect. Recent studies have found that chemicals that target the same signaling pathway or tissue elicit dose additive toxicity regardless of their specific mechanisms of action. Nevertheless, further studies are necessary to determine if this is true for other modes of action. Since anti-hyperlipidemic agents (as well as specific phthalates, haloacetic acids and/or PFAAs) have been implicated as developmental toxicants, the potential toxicological interactive response could be assessed in a short-term developmental toxicity paradigm.

Specific Aims

1. We propose to conduct dose-response studies with selected chemicals (2-4) representing the different mechanistic classes (e.g. modulators of cholesterol synthesis/uptake and utilization) to characterize the shape of each respective dose-response curve for developmental toxicity and will provide hazard assessment data on individual agents. This may include "window dosing" during critical periods of development.
2. Data from Aim 1 will be used to make toxicity predictions of the mixtures using dose addition, response addition, and integrated addition models.
3. Multiple mixture studies, both within a class (i.e. 3 or 4) and across classes (1 to 2 from each class; multiple classes), will be conducted to test the predictions made in Aim 2. This will provide hazard assessment data on mixtures.

Significance and Expected Outcome

Risk assessments have typically been performed on a chemical by chemical basis. However, real world exposures are to multiple chemicals, some with similar modes of action/targets. We will be developing prediction models for mixture-induced adverse developmental effects based on dose-response and potency estimates and test them experimentally. These data would expand our knowledge base on mixture toxicity and would explore the utility and predictivity of these models. Furthermore, these studies may identify potential toxicological concerns resulting from "additive" toxicities from multiple low dose level exposures of each agent. Data sets obtained from hypothesis-driven studies will be more easily generalized to assessment of risks from mixtures of varying composition (e.g. pharmaceuticals and/or environmental contaminants, including members of the groups that are not directly studied).

A better understanding of the toxicological challenges of mixture studies, including interpretation of the potential contribution of each mixture component, will likely aid in the design of future mixture studies be it from environmental exposure or from pharmacotherapy; as well as pharmaceutical combination toxicology studies when investigating the potential for interaction of chemicals with complementary modes of action.

Lipid and steroid modulating drugs as well as specific water contaminants have been shown to affect fetal development. However, little is known about the potential effects of altered lipid and steroid levels on fetal development. The data from these studies would broaden our understanding of the risks of concomitant exposure to these chemicals.

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